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RETARDATION OF THE DEVELOPMENT OF  
THE OVA OF THE HERRING

(WITH 1 PLATE).

BY

H. C. WILLIAMSON, M.A., D.Sc., F.R.S.E.

*This Paper may be referred to as :*  
*"Fisheries, Scotland, Sci. Invest., 1910, II. (Sept. 1911)."*



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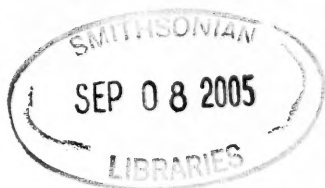
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# FISHERY BOARD FOR SCOTLAND.

## REPORT ON THE RETARDATION OF THE DEVELOPMENT OF THE OVA OF THE HERRING.

BY

H. CHAS. WILLIAMSON, M.A., D.Sc., F.R.S.E.,  
MARINE LABORATORY, ABERDEEN.

(Plate I.)

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The experiments on the retardation of the herring, which have been carried on at the request of the Government of New Zealand, were continued during the spring of 1911.

The eggs were fertilized on board a fishing boat on the evening of 27th February and the morning of the 29th February. The spawn was obtained from live herrings caught in a drift net. It was attached to glass plates and also to coarse gravel, as it was desired to see how the two methods compared in respect to the well-being of the ova. The method adopted in the case of the glass plates was similar to that described in my previous paper.\* For the second method a layer of gravel was put on the bottom of a barrel partly filled with sea-water. Some milt was pressed out into the water, and then some spawn was pressed out and allowed to fall on to the gravel. The gravel was left undisturbed for some hours. Twelve hours or more later, the barrel, on arrival at Aberdeen, was tipped up and the gravel turned out into a tub. A considerable quantity of the ova

\* “Experiments to show the Influence of Cold in retarding the Development of the Eggs of the Herring (*Clupea harengus*, L.), Plaice (*Pleuronectes platessa*, L.), and Haddock (*Gadus aeglefinus*, L.).” *Twenty-seventh Annual Report of the Fishery Board for Scotland*, Part III., 1910, p. 100.

was not attached to the gravel. Some eggs had been killed. A fair amount of ova was still adhering to the stones. They hatched out well, as did some of the eggs which were found loose among the gravel. The spawn was divided up. Part of both lots was cooled, while the remainder was kept in uncooled water. The glass plates, both cooled and uncooled, were made to revolve. The spawn on gravel, both cooled and uncooled, was put into trays, which were moved vertically. The revolution of the plates, and the raising and depressing of the trays, was intermittent, not continuous. The trays were made of wood, and they had bottoms sparred with glass tubes, and in one case covered with old galvanized wire netting.

The uncooled glass plates were put into the apparatus used in the 1910 experiment.\* It consisted of a galvanized iron box, which revolved inside a galvanized iron tank.

The apparatus used for the cooled spawn was of wood, most of which was tarred. The box containing the plates was 21 inches square and 18 inches deep (A., fig. 2). A wooden frame, which carried a plate on each of its four external faces, revolved on iron axles. Each plate was in succession brought to meet the inflowing current of water.

In the case of the uncooled gravel spawn the trays were raised a little and then depressed. The cooled gravel spawn was arranged in trays that travelled round on a revolving frame (B., fig. 2). The trays were 9 inches by 7 inches by 2 inches deep.

The water was filtered through a barrel filled with sand, both for cooled and uncooled ova.

For cooling the water a series of galvanized iron pipes ( $\frac{3}{4}$ -inch wide diameter), covered with ice, was employed.

Sea-water had been running through the pipes and the apparatus for three months before the ova were introduced.

The spawn was put into running water by about 10 p.m. on the 28th February.

The temperature of the uncooled and cooled water, and the quantity of water supplied to several of the lots of eggs, are shown in the table, p. 5. The temperature in °C. was read almost hourly during the day and night. The maximum and minimum thermometers were read once, sometimes twice, a day. The temperatures given in the maximum and minimum columns refer to parts of two days. The quantity of water is indicated by the time required to fill a one-pint measure (= .6 litre).

An estimate was made of the number of eggs on two plates, which were not too crowded with ova. They contained approximately 2600 and 2800 eggs respectively.

#### RATE OF MOVEMENT OF THE APPARATUS.

The cooled glass plates revolved once in from 1 to 2 minutes. The cooled gravel in trays made a revolution in from 5 to  $10\frac{1}{2}$  minutes. The plates in the tin revolved once in from  $1\frac{1}{2}$  to 2 minutes. The uncooled gravel spawn in trays was raised and depressed once in  $5\frac{1}{2}$  to  $7\frac{1}{2}$  minutes.

\* "Experiment in retarding the Development of the Eggs of the Herring." *Twenty-eighth Annual Report of the Fishery Board for Scotland, Part III., 1911.*

INCUBATION OF HERRING OVA—TEMPERATURE OF THE WATER, ETC.

| Number of Days. | TEMPERATURE OF THE WATER. |                      |                         |                      |                      | FLOW OF WATER.*  |        |                     |
|-----------------|---------------------------|----------------------|-------------------------|----------------------|----------------------|------------------|--------|---------------------|
|                 | (1)<br>Gravel.            |                      | (2)<br>Glass<br>Plates. | (3)<br>Tin.          | Un-<br>cooled.       |                  |        |                     |
|                 | Range in<br>°C.           | Max. and<br>Min. °F. | Max. and<br>Min. °F.    | Max. and<br>Min. °F. | Max. and<br>Min. °F. | Nos.<br>1 and 2. | Tin.   | Uncool'd<br>Gravel. |
|                 |                           |                      |                         |                      |                      | Secs.            | Secs.  | Secs.               |
| 1               | 3-2.6                     | 42, 40               | 43, 40                  | 42.5, 40             | 42.5, 40             | 13               | ..     | ..                  |
| 2               | 3.4-2.6                   | 42, 37               | 42, 37                  | 42.5, 41             | 42.5, 41             | 14               | 22     | 95                  |
| 3               | 3.2-2                     | 38, 36               | 40, 36                  | 44.5, 43             | 44.5, 43             | 18               | ..     | ..                  |
| 4               | 4-1.6                     | 38, 36               | 40, 36                  | 42.5                 | 42.5                 | 13-24            | 22     | 20                  |
| 5               | 2.5-1.6                   | 38, 35               | 40, 35                  | 44.5, 39             | 44.5, 39             | 17               | ..     | ..                  |
| 6               | 2.6-1.6                   | 36, 35               | 38, 35                  | 41.5, 41             | 41.5, 41             | 21               | ..     | ..                  |
| 7               | 2.2-1.4                   | 36, 35               | 37, 36                  | 41.5, 40             | 41.5, 40             | 9-21             | ..     | ..                  |
| 8               | 2.1-1.4                   | 35+, 35              | 36, 36                  | 39.5                 | 39.5                 | 9-1              | 21, 30 | 14, 18              |
| 9               | 1.9-1.4                   | 35+, 35              | 36, 35                  | 42.5, 40             | 42.5, 40             | 18               | 22     | 17                  |
| 10              | 1.9-1.6                   | 37, 35               | 36, 35                  | 42.5, 39             | 42.5, 39             | 16, 21           | 23     | 18                  |
| 11              | 1.9-1.4                   | 35, 35               | 35, 35                  | 42.5, 39             | 42.5, 39             | 16, 22           | 27     | 20                  |
| 12              | 1.9-1.4                   | 35, 34.5             | 36, 35                  | 41.5, 39             | 41.5, 39             | 14-26            | 22     | ..                  |
| 13              | 1.8+-1.4                  | 35, 34               | 35.5, 34.5              | 40.5, 39             | 40.5, 39             | 13-18            | 23     | 38                  |
| 14              | 2.2-1.3                   | 36, 34.5             | 36, 34.5                | ..                   | ..                   | 10-26            | 30     | 75                  |
| 15              | 2-1.4                     | 36, 34.5             | 36, 35                  | ..                   | ..                   | 14-23            | 25     | ..                  |
| 16              | 2.4-1.6                   | 36, 35               | 36, 35.5                | 40.5, 38             | 40.5, 38             | 13-18            | 44     | 46                  |
| 17              | 2.2-1.7                   | 36, 35               | 36, 35.5                | ..                   | ..                   | 11-27            | 18-46  | 10                  |
| 18              | 2-1.6                     | 35.5, 35             | 36, 35.5                | 41.5, 40             | 41.5, 40             | 13, 17           | 19     | 10, 29              |
| 19              | 2-1.4                     | 35.2, 34.5           | 35.5, 35                | ..                   | ..                   | 10-15            | ..     | ..                  |
| 20              | 1.7-1.4                   | 35.2, 34.5           | 35.5, 35                | ..                   | ..                   | 11-16            | 19     | 31                  |
| 21              | 1.9-1.3                   | 35, 35               | 35.5, 35                | 40.5, 37             | 41.5, 39             | 18               | ..     | ..                  |
| 22              | 2-1.6                     | 35.2, 35             | 35.5, 35                | 36, 35               | ..                   | 13-18            | ..     | ..                  |
| 23              | 1.8-1.6                   | 35.2, 34.5           | 35.5, 35                | 37                   | 41.5, 40             | 14-18            | 100    | 32                  |
| 24              | 2-1.4                     | 35.2, 34.5           | 35.5, 35                | 37, 35               | 39.5, 39             | 17               | 35     | 30                  |
| 25              | 2-1.4                     | 35.2, 34.5           | 35.2, 35                | 37, 35.2             | 42                   | 19               | 34     | 32                  |
| 26              | 1.8-1.4                   | 35.5, 34.5           | 36, 35                  | 37, 35               | ..                   | 16               | 25     | ..                  |
| 27              | 1.9-1.4                   | 35.5, 34.5           | 36, 35                  | 37, 35               | 42.5, 40             | 16               | 40     | ..                  |
| 28              | 2-1.4                     | 35, 34.5             | 35.5, 35                | 37, 36               | 42.5, 41             | 18, 20           | ..     | ..                  |
| 29              | 1.9-1.6                   | 35, 35               | 36, 35.5                | 37, 36               | 42.5, 41             | 20               | 65     | ..                  |
| 30              | 1.9-1.6                   | 35, 34.5             | 36, 35.5                | 37, 36               | ..                   | 15-23            | 55     | ..                  |
| 31              | 2-1.6                     | 35.2, 34.5           | 36, 35.5                | 37.5, 36             | ..                   | 15-32            | 75     | ..                  |
| 32              | 2-1.4                     | 35.2, 34.5           | 36, 35.5                | 37.5, 36             | 43.5, 41             | 16-28            | 65     | ..                  |
| 33              | 2-1.4                     | 35.2, 34.5           | 35.5, 35                | 37, 36               | 42.5, 41             | 17-26            | ..     | ..                  |
| 34              | 1.9-1.2                   | 35, 34               | 35.5, 34.5              | 38, 35               | 42.5, 40             | 15, 28           | ..     | ..                  |
| 35              | 1.8-1.4                   | 35, 34.2             | 35.5, 34.5              | 37, 35               | 41.5, 39             | 13-17            | 63     | ..                  |
| 36              | 1.8-1.2                   | 35, 34.2             | 35.5, 34.5              | 37, 35               | 40.5, 39             | 14-17            | 63     | ..                  |
| 37              | 1.8-1.2                   | 35, 34               | 35.5, 34.5              | 36, 35               | 39.5, 38             | 14-30            | 65     | ..                  |
| 38              | 1.8-1.6                   | 35, 35               | 36, 35.5                | 37.5, 35             | 42.5, 39             | 15-30            | ..     | ..                  |
| 39              | 1.9-1.3                   | 35.2, 34.2           | 36, 35                  | 37, 37               | 42.5, 42             | 15-25            | ..     | ..                  |
| 40              | 2-1.4                     | 35, 35               | 36, 35                  | 37, 37               | ..                   | 20               | ..     | ..                  |
| 41              | 1.8-1.2                   | 35.2, 34.2           | 36, 34.5                | 38, 36               | 44.5, 42             | 15-25            | ..     | ..                  |
| 42              | 2.1-1.4                   | 35.5, 34.2           | 36, 35                  | ..                   | 42.5, 39             | 15, 17           | ..     | ..                  |
| 43              | 1.9-1.4                   | 35.5, 34.2           | 36, 35.2                | ..                   | ..                   | 17-26            | 63     | ..                  |
| 44              | 2-1.4                     | 35.5, 34.2           | 35.5, 35                | ..                   | 44.5, 42             | 18-25            | ..     | ..                  |

\* The quantity of the flow of water is indicated by the time required to fill a vessel of a capacity of 1 pint (=·6 litre).



INCUBATION OF HERRING OVA—*continued*.

| Number of Days. | TEMPERATURE OF THE WATER. |                      |                         |                      |                      | FLOW OF WATER.   |       |                     |
|-----------------|---------------------------|----------------------|-------------------------|----------------------|----------------------|------------------|-------|---------------------|
|                 | (1)<br>Gravel.            |                      | (2)<br>Glass<br>Plates. | (3)<br>Tin.          | Un-<br>cooled.       |                  |       |                     |
|                 | Range in<br>°C.           | Max. and<br>Min. °F. | Max. and<br>Min. °F.    | Max. and<br>Min. °F. | Max. and<br>Min. °F. | Nos.<br>1 and 2. | Tin.  | Uncool'd<br>Gravel. |
|                 |                           |                      |                         |                      |                      | Secs.            | Secs. | Secs.               |
| 45              | 2.2—1.4                   | 35, 35               | 36.5, 35.5              | ..                   | :                    | 15—23            | ..    | ..                  |
| 46              | 2—1.2                     | 35.5, 34.2           | 36, 35.5                | ..                   | ..                   | *17, 19          | ..    | ..                  |
| 47              | 2—1                       | 35.2, 34             | 36, 34.2                | ..                   | 45.5, 42             | 14, 18           | ..    | ..                  |
| 48              | 1.8—1                     | 35, 34               | 35.5, 34.2              | ..                   | 44.5, 38             | 18               | ..    | ..                  |
| 49              | 2—1.2                     | 35, 34               | 36, 35                  | ..                   | 43.5, 42             | 16—27            | ..    | ..                  |
| 50              | 2.2—1.2                   | 35.5, 34.2           | 36, 35.5                | ..                   | 45.5, 44             | 17, 36           | ..    | ..                  |
| 51              | 6.8                       | 44, 36               | 44, 66                  | ..                   | 46, 44               | 9                | ..    | ..                  |
| 52              | ..                        | ..                   | ..                      | ..                   | 44.5, 43.5           | ..               | ..    | ..                  |
| 53              | ..                        | ..                   | ..                      | ..                   | 47, 43               | ..               | ..    | ..                  |
| 54              | ..                        | ..                   | ..                      | ..                   | 49, 47               | ..               | ..    | ..                  |
| 55              | ..                        | ..                   | ..                      | ..                   | 48.5, 48.5           | ..               | ..    | ..                  |

\* Water supply stopped for some time during the day.

## RESULTS.

*The Uncooled Spawn on Gravel.*

This spawn was not very clean.

During incubation, the spawn was fully exposed to daylight, but not to direct sunlight. The eggs detached from the gravel varied in size from 1.32 to 1.5 mm. in diameter. Eggs which had been stuck to the gravel, and which had been forcibly detached, had lost a patch of the outer layer of the zona. This did not appear to exercise any ill effect. No crystals were observed in this lot of spawn.

The first larva appeared on the 19th day, and larvæ were obtained almost daily up to the 29th day. On the latter date a few of the eggs still contained embryos.

*Spawn Incubated in the Galvanized Tin.*

This lot of spawn was treated with water similar to that supplied to the uncooled gravel spawn up till the time when the larvæ began to appear, viz., on the 20th day. Thereafter the water was cooled, and the incubation-period was, in consequence, extended for an additional 21 days. The larvæ hatched out daily from the 20th to the 41st day. The results in this case were very satisfactory.

The retardation consisted in keeping the developed embryos in the egg after they were ready to hatch. The result was that many of the larvæ, when they hatched, had already consumed a large portion of the yolk. On the 40th day three had only a trace of yolk left (fig. 6), although a fourth had still a fairly large ball of yolk unabsorbed. A normally hatched larva may be 8 mm. in length: a post-larva measured 7.5 or 8 mm. The eggs did not seem to have suffered from the cooling.



Diatoms were noticed at different times on the eggs, some of which became somewhat dirty externally. They were, however, partly sheltered from the light by being enclosed within the partially-closed tin. They did not, therefore, become so dirty with diatomaceous growth as the other lots of eggs.

Crystals, in some cases in great quantity, were observed in certain eggs. The majority showed none. The crystals were attached to the zona, sometimes in rosette-form, or even inside the embryo. They were observed in one of the trunk canals, apparently the gut (fig. 3). In the embryo, a large corpuscle plugged the heart with each pulsation, and then receded again (fig. 9). Next day the corpuscle remained clear of the heart. Crystals were observed in the gut of another embryo, the heart of which had no plug. One lively embryo had a huge quantity of crystals attached to the inside of the zona.

#### *Cooled Spawn.*

The gravel spawn examined nine days after spawning seemed to be in a pretty good condition, but some dead eggs were to be seen. On the 11th day, the spawn on the glass plates looked well, except where the eggs were in a thick mass. In such places dead eggs were observed. Some of them had no doubt been killed by the pressure of adjacent eggs. Certain eggs were of especially large diameter. Three that contained embryos showed no crystals, but in certain of them a granular matter was sticking on the embryo—an unhealthy sign. An egg, 1.75 in diameter, had an unhealthy looking embryo. Two eggs measured 1.3 and 1.35 mm. in diameter. Both contained crystals. In one the crystals were large and few in number; in the other they were small and fairly numerous.

By the 22nd day a copious growth of diatoms was noticed on the eggs. The thickly-covered plates did not look so well as those that had a sparse coating of eggs. Larvæ began to appear on the 29th day. On the 42nd day a considerable quantity of dead eggs was observed. Many appeared to the naked eye of a milky tinge. That probably indicates that they had died recently. The milky appearance is due to the perivitelline fluid turning opaque. Some of the ova were yellow-coloured from the coating of diatoms.

On the 51st day, ripe eggs, containing live embryos, were quite yellowish. A good proportion of the eggs of one of the good plates seemed to have hatched.

I examined some eggs that had died recently. In one I could detect no movement of the embryo. I dissected it out of the egg capsule. The heart was found to be beating slowly. The embryo seemed to be perfect. It was, I think, dying from suffocation, due to the mat of diatoms that covered the zona. The larvæ which were obtained on each day from the 29th to the 38th day were prematurely hatched. They were very small, viz., 4.5–6 mm. long. The head was markedly bent downwards, *i.e.*, much more than in the older larvæ. The postlarval body is shorter than normal. Succeeding batches gradually improved in size. On the 38th day 94 were obtained. Compared with a larva which had consumed nearly all its yolk, they were shorter and had the head more flexed. They were pretty lively. Two measured 6 and 7.5 mm. in length respectively. Thereafter the

following quantities of fry were obtained :—39th day, 120 (20 dead)\*; 40th, 250 (20 dead); 41st, 191; 42nd, 335 (100 dead); 43rd, 160 (10 dead); 44th, 187 (10 dead); 45th, 98 (4 dead); 46th, 67 (8 dead); 47th, 24 (2 dead); 48th, 27 (1 dead); 49th, 16 (5 dead); 50th, 14 (4 dead); 51st, 8 (3 dead); 52nd, 1; 53rd, 1 larva.

After the 39th day the larvæ seemed normal. They were shorter on the whole than the average larva, and they had a comparatively large amount of yolk still remaining unabsorbed. The heads were flexed. They wriggled about quite actively with eel-like movement. On the 47th day two measured 7 and 8 mm. respectively. The head was only slightly flexed. On the 50th day 10 good fry showed still a good quantity of yolk. The heads were slightly flexed.

The hatching of the ova began prematurely in consequence, I consider, of the decay of the zona, caused by the diatoms attached to it. The same cause led, in my opinion, to the death, through suffocation, of many ova. There was also a large quantity of infusors and many nematodes among the eggs.

The larvæ do not appear to have developed much too rapidly. The cooling was doubtless not sufficiently low. I think a slightly lower temperature, 34° to 35° F., would not injure the ova and would probably be sufficient to retard the ova for the requisite period.

#### *Gravel and Glass.*

While the spawn attached to the gravel did very well, that on the glass could not be said to be distinctly inferior. Glass plates are much more easily handled and with their aid a larger quantity of ova can be dealt with. The spawn on the gravel was less in quantity, less crowded, and its situation on small pieces of stone permitted, possibly, a more effective aeration.

#### *Exposure to Light: Growth of Diatoms.*

A danger which was apparent during this experiment has, I believe, a greater bearing on the well-being of the ova than their location on glass or gravel. That is, exposure to light. Light has a great influence in stimulating the growth of diatoms as Allant† and Nelson show. If deprived of light the culture of diatoms dies off. The quality of the water also affects the growth of diatoms. The two authors point out that in the tank-water of the Plymouth Laboratory larger and healthier growths of diatoms were got than in water procured some distance off shore. This fact was ascribed to the greater quantity of organic matter in the tank-water.

The diatoms which grow on the ova tend to prevent the aeration of the embryo, and are also, I consider, the probable cause of the premature escape of the larvæ by hastening the decay of the zona. No doubt decay takes place normally during incubation. If that decay be hastened the embryo may be able to burst its way to freedom at a smaller size than the average.

\* The larvæ were caught in a trap in which some were killed.

† "The Artificial Culture of Marine Plankton Organisms." *Quarterly Journal of Microscopical Science*, Vol. 55, Pl. 2. June, 1910.

Diatoms attached to the eggs of Lobster, exposed to bright light, caused the decay of the outer egg-membrane. (Anderton) *Report of Marine Department, New Zealand*, 1908-9. Wellington, 1909.

*The Current of Water.*

I think that the current of water should be strong. It is not necessary that it be continuous: it may be intermittent. A large quantity sent through the apparatus at intervals would probably be more effective than a small continuous current.

*The Filter-Barrel.*

During the experiment, the filter-barrel, in which was filtered the water that was cooled, was allowed to work for 40 days. This was probably an error. Whether it affected the success of the experiment is doubtful. It would be better to have the filters frequently cleaned.

EXPERIMENTS WITH SPAWN FROM LIVE AND DEAD HERRINGS.

*On Galvanized Wire Gauze and Glass Plates.*

A number of ripe herrings were put into a herring barrel and sent to Aberdeen. On arrival all but two were dead. The survivors were a male and a female. The herrings had been probably not more than 12 hours in the barrel. Four experiments were carried out on March 24th 1911.

(A) Some spawn was obtained from the two live fishes and it was put on a piece of galvanized wire gauze. The gauze was new, but it had been for a day or two in running sea-water. The eggs appeared to do well for a few days. They showed lots of crystals. The eggs gradually died off, and on the 25th day after fertilization all but three were dead. The embryo at that time showed black pigment in the eyes.

(B) Spawn and milt obtained from dead herrings were put on narrow-meshed galvanized wire gauze in hatchery water. The eggs developed for a time, but by the 20th day all but one or two were dead.

(C) Spawn was obtained from a dead fish and put on a glass plate. This spawn was put into the water in which the herring travelled to Aberdeen. No fresh milt was supplied to the water. I examined a drop of the water: tailed sperms were visible, but they showed little motion. Fertilization ensued, however. Although most of the eggs died off, two which had embryos ready to hatch were found alive on the 31st day. A good number of the eggs had died after pigment had begun to show in the eyes of the embryo.

(D) Some spawn and milt were taken from dead herrings and put on a glass plate in new (hatchery) water. Fertilization took place in this case also. Many of the eggs had well developed embryos, but most died before hatching.

The four lots of spawn stood, after being prepared, for some hours in still water. They were then all put in a revolving tin box set in a galvanized iron tank. The water supplied to them was cooled. The temperature is given in the column marked "Tin" (p. 5) from the date March 26th onwards. One egg which had been in still water all night showed a large normally segmented disc and a huge quantity of crystals. Some fry were got from the spawn on the 26th to 31 days after fertilization.

*Milt and Spawn.*

Some milt from a dead herring was put into sea-water. I found the sperms (fig. 7) oscillating vigorously as if anchored by the tail. Many, however, oscillated very faintly.

No difference was observed between the spawn of the living and dead herrings.

Some unfertilized eggs, a quarter of an hour after they had been deposited on the glass plate, exhibited different shapes (fig. 8). Most of them show an inpushing where they had been pressed by another egg. No sign of perivitelline space was visible. The eggs were easily dislodged from the glass. They do not adhere strongly until the perivitelline space is formed. Three days later these unfertilized eggs had a fairly large perivitelline space, but they remained dull tinted on the outside, instead of showing a clear translucent zona.

## THE CRYSTALS IN THE EGGS.

The crystals, which were very evident during the two previous experiments, were found also on this occasion. They are rectangular (fig. 5), sometimes ending in a tapering oblique point. They may be fine, needle-like, or fairly thick bars. They appeared in the batches of eggs which were exposed to the influence of galvanized iron. In one instance, they appeared within 36 hours after fertilization (fig. 10). This egg had been in still water in an enamelled bath for a night, and had probably with it some galvanized gauze.

No crystals were observed in the uncooled eggs on gravel. The water was delivered to these eggs through large galvanized iron pipes, which had been in use for several years. All the cooled spawn received water through a series of small galvanized iron pipes.

One remarkable fact is that the crystals were not present in all the eggs of a batch. They may be found (1) on the inside of the zona; (2) between the layers of the zona; (3) attached to the yolk or to the embryo; and (4) within the embryo.

These crystals are soluble in acetic acid, and in sodium hydrate solution. They are insoluble in water, alcohol, and methylic ether. Dr. J. K. Wood, Chemical Department, University College, Dundee, says that the crystals, being able to act both as a base and an acid, are probably formed from proteids by some process of decomposition.

I have come to regard the galvanized iron as, in some measure, the cause of the formation of these crystals. The galvanized iron becomes coated with a white incrustation in sea-water. Dr. Wood was not able to find zinc in solution in the sea-water in which a piece of this metal had been lying for several days. It seems possible that the cause of the formation of the crystals is to be found in electrolytic action. The electric current acting within the eggs on the fluids containing excretory products causes the crystallization. I formerly regarded the crystals as indicative of deficient aeration, and I still favour that view. The deficient aeration resulting in the accumulation of excretory products in the perivitelline fluid may afford the opportunity for the action of the current. All the eggs do not exhibit the crystals. It is possible that the zona may not be acting satisfactorily from the point of view of osmosis. It is possible that variation may

occur in that capsule. As I pointed out above, the diameter of the zona varies much. In some eggs the two layers of the zona may be seen separated. In one egg here and there the vitelline membrane may be made out. Further, the eggs are differently served by the currents of water.

It is quite possible that the quality of the water at the Laboratory may be a contributory factor. It is probably charged with organic matter.

While active embryos are found in eggs containing the crystals, it is not likely that their presence is an advantage. When the crystals are formed in a canal in the embryo, they will almost surely prove a fatal encumbrance.

#### RECOMMENDATIONS.

If the experiment of transporting the ova of the herring to New Zealand be made, the following would, I think, be a suitable apparatus. It is shown in plan in fig. 1. It consists of six compartments, each of which contains a revolving frame. The frame will be able to carry on its external faces four glass plates, 9 inches by 7 inches. A section through two of the compartments is given in fig. 1A. A shaft running longitudinally above the middle of the apparatus would impart the revolving motion to the frames by means of little belts (rubber cord.)

The amount of water circulated through the apparatus should be as large as possible. If iron pipes are used to cool the water it will probably be better to have ordinary unprotected iron pipes.\* In any case, the pipes and apparatus should be well seasoned, by being exposed to the action of sea-water, for some time previous to the experiment being made. The water when it leaves the cooling apparatus should pass into a reservoir, surrounded by ice. Thence it should flow through the apparatus. If the water be not used over again, the waste water can be utilized to cool the incoming water. Where the water is to be used over again, a pump will be required to bring it from the reservoir. A small quantity of freshly-cooled water should be steadily added.

This apparatus should be provided with lids, and should be kept in a dimly-lighted apartment.

It is most important to have clean water at all times for the spawn. This should be carefully arranged when the spawning is taking place, for mud, etc., adhere readily to the ova. Wherever necessary, the water should be filtered through sand. It is hardly possible that all the eggs pressed on to the plates will live.

Where only a small quantity of cooled water is available, it might be advisable to have it sterilized. I have not, however, made any experiments with sterilized water.

Some precautions should be observed when the spawn is being obtained. Only spawn that runs freely on gentle pressure should be employed. Milt and spawn should be preferably obtained from living fishes. The eggs should be arranged thinly on the plate. They should be protected from being touched after they are on the plate. The spawn should be brought into moving water as soon as possible.

\* *Vide* Davis., "The Action of Water on Zinc and Galvanized Iron." *The Journal of Chemical Industry*. Vol. XVIII., Feb. 28, 1899.

An interval of four to six hours in quiescent water has, however, usually been given to allow of the ova becoming fastened to the glass.

The glass plates can be put into wooden boxes, each holding about six plates. The boxes may be put in a herring barrel into a frame, which can be revolved from time to time. The barrel would, if necessary, be surrounded with ice during the railway journey. The spawn should be protected from the light.

For the woodwork, I think coal-tar is a suitable preservative.

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### PLATE I.

FIG. 1. Drawing (plan) of apparatus for conveyance of herring spawn.

- „ 1A. „ (section) „ „
  - „ 2. Drawing of apparatus in which the cooled herring spawn was kept during the Spring of 1911.
  - „ 3. Enlarged drawing of part of embryo, to show crystals in gut.
  - „ 4. Live egg attached to dead egg, showing large number of crystals.
  - „ 5. Enlarged drawing of crystal.
  - „ 6. Enlarged drawing of anterior end of a larva, which had been retained in the egg until much of the yolk had been absorbed.
  - „ 7. Enlarged drawing of a sperm.
  - „ 8. Different shapes assumed by unfertilized eggs when they fell on the glass plate.
  - „ 9. Drawing to show the corpuscle which alternately blocked and receded from the heart of the embryo partially shown in fig. 3.
  - „ 10. Egg in which crystals had appeared within 36 hours of spawning.
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### LETTERS USED.

*a.*—Anus.  
*cr.*—Crystal.  
*de.*—Dead egg,  
*dmf.*—Dorsal marginal fin.  
*gt.*—Gut.  
*le.*—Live egg.

*nt.*—Notochord.  
*o.*—Outer skin of zona.  
*pl.*—Glass plate.  
*t.*—Tray.  
*vmf.*—Ventral marginal fin.  
*yk.*—Yolk.







[illegible]





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